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Naturalistic Experimental Designs as Tools for Understanding the Role of Genes and the Environment in Prevention Research

Leslie D. Leve,[1] Jenae M. Neiderhiser,[2] Gordon T. Harold,[3,4] Misaki N. Natsuaki,[5] Brendan J. M. Bohannan,[1] William A. Cresko[1]

[1]University of Oregon; [2]The Pennsylvania State University; [3]University of Sussex; [4]Tomsk State University; [5]Universiy of California, Riverside

Correspondence concerning this article should be addressed to Leslie Leve, PhD., Prevention Science Institute, 6217 University of Oregon, Eugene, OR, 97403-6217. E-mail: leve@uoregon.edu.

Abstract

Before genetic approaches were applied in experimental studies with human populations, they were used by animal and plant breeders to observe, and experimentally manipulate, the role of genes and environment on specific phenotypic or behavioral outcomes. For obvious ethical reasons, the same level of experimental control is not possible in human populations. Nonetheless, there are natural experimental designs in human populations that can serve as logical extensions of the rigorous quantitative genetic experimental designs used by animal and plant researchers. Applying concepts such as cross-fostering and common garden rearing approaches from the life science discipline, we describe human designs that can serve as naturalistic proxies for the controlled quantitative genetic experiments facilitated in life sciences research. We present the prevention relevance of three such human designs: (1) children adopted at birth by parents to whom they are not genetically related (common garden approach); (2) sibling designs where one sibling is reared from birth with unrelated adoptive parents and the other sibling is reared from birth by the biological mother of the sibling pair (cross-fostering approach); and (3) in vitro fertilization designs, including egg donation, sperm donation, embryo donation, and surrogacy (prenatal cross-fostering approach). Each of these designs allows for differentiation of the effects of the prenatal and/or postnatal rearing environment from effects of genes shared between parent and child in naturalistic ways that can inform prevention efforts. Example findings from each design type are provided and conclusions drawn about the relevance of naturalistic genetic designs to prevention science.

Keywords: genetic, environment, cross-fostering, adoption, intervention

Naturalistic Experimental Designs as Tools for Understanding the Role of Genes and the Environment in Prevention Research

Inquiry about the inheritance of behavior has a long history, beginning in the 19th century with the publication of seminal works by Darwin on the '*Origin of Species*' (Darwin, 1859), by Mendel (1863) on breeding studies of pea plants, and by Galton (1883) on family studies demonstrating that behavioral characteristics run in families (see supplementary materials for a more detailed summary of the historical context). Fisher (1918) then spawned the field of quantitative genetics, using Mendelian genetics to dissect the resemblance among individuals of varying relatedness, and laying the groundwork for population level statistics of breeding values, additive genetic variance, and heritability that are used in genetics to this day (Mackay, 2001).

Quantitative genetics was first adopted and expanded by plant and animal breeders to guide the improvement of stocks through breeding and artificial selection (Falconer & Mackay, 1996). They developed the most common experimental designs for quantitative genetic analyses, an influence clearly seen in the quantitative genetic terminology. For example, a 'common garden' approach is used to reduce the environmental variance and more clearly reveal genetic differences (MacKay, 2001). Similarly, controlled breeding such as the 'half sibling' design can create a range of relatedness among individuals that can be used to estimate quantitative genetic parameters (Zeng, Kao, & Basten, 1999).

It is these early beginnings, starting with Darwin's work over 150 years ago, from which the modern field of human behavioral genetics emerged. More recently, advances in molecular genetic technologies have allowed the linking of particular genomic regions to a quantitative trait (Quantitative Trait Loci; QTLs) that can be applied in breeding designs or in Genome Wide Association Studies (GWAS) in outbred populations (Sale, Mychaleckyj, & Chen, 2009). Many

of the approaches used in experimental designs by plant and animal breeders can be applied to natural pedigree studies in humans. Building on the experimental designs of plant and animal genetics, we describe how naturalistic experiments made possible by modern day family constellations can advance our understanding of the role of genetic and environmental influences on child psychopathology. We focus on naturalistic experimental designs that mirror those originally used in controlled pedigree experiments in the life sciences, with an emphasis on the translation of findings to prevention science. First, we describe key concepts related to gene-environment interplay in quantitative genetic designs with human populations. Next, we describe three naturalistic experimental designs (the parent-offspring adoption design, the siblings reared apart design, and the *in vitro* fertilization design; See Figure 1) and provide examples of research findings from each that can inform the design of preventive interventions. Although other naturalistic designs also may inform prevention research (e.g., discordant twin designs, step-family designs, or children of twins designs), we limit our focus to three designs due to space considerations. We conclude by highlighting microbiome studies as a recent genetic approach that could prove highly relevant for the promotion of child health and well-being.

Gene-Environment Interaction and Correlation and Their Relevance to Prevention

Two forms of gene-environment interplay are particularly relevant to prevention science and to the three naturalistic experimental designs described in this manuscript: gene x environment interaction (GxE) and gene-environment correlation (*r*GE). In one form of GxE, psychopathology may result from an inherited liability to disorder (diathesis) that is manifested under particular (stress-inducing) environmental conditions. This form of GxE is consistent with diathesis-stress models of development. The literature has many examples of diathesis-stress forms of GxE from parent-offspring adoption studies (e.g., Cadoret et al., 1995) and twin studies

(e.g., Kendler et al., 1995). For example, an adoption study showed that infants whose birth mother showed elevated levels of antisocial behavior were more sensitive to the stress of living with a depressed mother (Leve et al., 2010). A second form of GxE, differential susceptibility (or biological sensitivity to context), has been proposed more recently (Belsky & Pluess, 2009; Ellis et al., 2011). Differential susceptibility theory postulates that heritable factors increase sensitivity to the environment, resulting in more benefit from favorable environments and more detriment from adverse environments. Empirical tests of differential susceptibility have provided some support for differential sensitivity to intervention (e.g., Brody et al., 2013) and differential responses to parenting (e.g., Dick et al., 2011). Together, studies examining GxE have identified specific environmental features that can offset or exacerbate genetic influences on psychopathology, thereby providing the groundwork for targeted interventions that can override or build upon heritable traits of an individual.

A second way in which genes and environment work together to affect child well-being is via gene-environment correlation (*r*GE). Accumulating evidence indicates that heritable qualities in children and adolescents influence their relationships with parents and peers (e.g., Horwitz & Neiderhiser, 2015). This process is termed evocative *r*GE, and it occurs when inherited characteristics in the child evoke a response from the environment. Two separate adoption studies have found evidence for evocative *r*GE in late childhood through late adolescence: in both studies, children whose biological parents were more antisocial were more likely to receive negative or hostile parenting from their adoptive parents than children without this inherited risk (Ge et al., 1996; O'Connor et al., 1998). Twin studies have also reported evidence of heritable qualities in children evoking parental behavior across a wide range of parenting constructs and child ages (see Klahr et al., 2014 for a meta-analysis).

Passive *r*GE is a second way in which children's inherited qualities can be correlated with their environment. Passive *r*GE occurs when genes are shared between a rearing parent and a child. Quantitative genetic studies have identified passive *r*GE influences during middle childhood (e.g., Lemery-Chalfant, et al., 2013) and adolescence (e.g., Neiderhiser, et al., 2007). Concerns that passive *r*GE confounds our understanding of intergenerational transmission has inspired designs like the children of twins (COT) design, which can control for passive *r*GE while also estimating direct environmental effects. Studies using the COT approach have found that the intergenerational transmission of anxiety symptoms, for example, is best explained by direct environmental effects, not passive *r*GE (Eley et al., 2015). Natural experimental designs that include genetically unrelated parent-child dyads eliminate the effects of passive *r*GE on associations between parent and child behavior, and therefore provide more direct estimates of environmental influences on child psychopathology that can be targeted in intervention research. The first naturalistic experimental design we present offers unique strengths in this regard: the full parent-offspring adoption design.

Naturalistic Experimental Design 1: The Full Parent-Offspring Adoption Design

The full parent-offspring adoption design in human populations shares a unique design feature with the "common garden" approach used in life sciences research: the environment is hypothesized to have an influence on individual units reared together in that environment, and its effects can be separated from genetic influences by restricting the variance in either the environment or in the genetic background of the individuals in that environment (see Figure 1). Whereas in plant research this refers to, for example, different seed variants grown in specific lighting, temperature, and/or water conditions that are hypothesized to affect plant health, in the parent-offspring adoption design, this refers to adopted children reared in a family environment

in which they are genetically-unrelated to the rearing parents. Adopted children who are placed with nonrelatives at birth are genetically unrelated to their rearing parents, yet share the family environment and the larger social context with their rearing parents. In the absence of systematic biases in the selection of adoptive families by birth parents or vice versa, similarities between an adopted child and his or her adoptive parents are best explained by shared environmental (or common environmental) influences. A full adoption design also includes birth parents in order to enable the estimation of inherited influences on development. Similarities between birth parents and the adopted child suggest genetic influences (due to shared genes and a lack of shared rearing environments). Similarities between adoptive parents and adopted children indicate environmental processes (due to shared rearing environments and the lack of shared genes), although evocative *r*GE effects may also cause parent–child similarities.

There have been two prospective full parent-offspring adoption studies that have followed children from around the time of birth for a decade or more. The first and landmark parent-child longitudinal adoption study of this type was the Colorado Adoption Project (CAP; Plomin & DeFries, 1983). Initiated in 1975, infants were placed with adoptive families when they were an average age of 29 days old. The final parent sample includes 286 birth mothers, 60 birth fathers, 242 adoptive mothers, and 237 adoptive fathers. In addition, a one-to-one matched control group of nonadoptive parents rearing their biological children was ascertained. Adoptees in CAP have been assessed at regular intervals from infancy into adulthood. The study has yielded evidence of increasing heritability on cognitive abilities from early childhood to adolescence (Rhea et al., 2013) and GxE interactions on social behaviors during later childhood (e.g., Hershberger, 1994) and has been instrumental in furthering the understanding of the role of the environment on development.

The Early Growth and Development Study. A second full parent-offspring adoption design study began 25 years later, with a primary focus on children's psychosocial development and the parenting environment. This study, the Early Growth and Development Study (EGDS), includes 561 linked sets of participants: 561 adopted children, their adoptive parents (552 adoptive fathers and 567 adoptive mothers; this includes 41 same-sex parent families), their birth mothers (n = 554), and their birth fathers (n = 208) (Leve et al, 2013). Participants were recruited from domestic adoption agencies across the United States. The median child age at adoption placement was 2 days (M = 6.2, SD = 12.45). In-person assessments are ongoing at regular intervals throughout the study, from at age 9 months to adolescence, and birth parent in-person assessments occurred three times between child age 4 months and 5 years.

Prevention Relevant Results. The EGDS dataset has been used to examine the interplay between genetic and environmental influences on a range of child outcomes related to the prevention of child psychopathology. In one study, associations between mother's structured parenting and toddler behavior problems were examined as a function of whether the child had an elevated inherited risk for psychopathology, as measured by birth parent psychopathology (e.g., anxiety, depression, antisocial behavior, and drug use) (Leve et al., 2009). Structured parenting was observed during a parent-child clean-up task and microsocially coded for maternal statements and questions that aimed to promote behavioral change or suggest a specific task-relevant action to the child, such as "Where does this ring go?," or "Put the duck in this box." Emotional tone was not coded and as such, the defining characteristic was whether the parent structured the task for the child (as opposed to engaging in other behaviors such as talking about non-task related topics, providing positive reinforcement, or ignoring the child). An interaction between inherited risk and maternal structured parenting was found, indicating two distinct

pathways to child problems: structured parenting was beneficial for toddlers at high inherited risk, but was related to more behavior problems for toddlers at low inherited risk. This finding has important prevention relevance because it suggests that a one-size fits all parenting intervention may not be beneficial for all children. When the sample is examined as a whole, without consideration of inherited risk, these divergent pathways to child behavior problems are masked. By using quantitative genetic methods and testing genetic moderation of environmental influences on development, the differing effects of high maternal structure on child behavior problems are identified. In this study, one implication is that consideration of the individual, inherited risks a toddler presents with might be warranted when implementing interventions aimed at preventing risk for psychopathology during early childhood.

Results from EGDS have also highlighted the specific mechanisms whereby inherited characteristics in very young children affect their rearing environment, which then affect child outcomes. In one study, evidence for evocative *r*GE processes from birth mother ADHD symptoms to adoptive mother hostile parenting through early disruptive child behavior at age 4.5 were identified (Harold et al., 2013). Further, maternal hostile parenting and disruptive child behavior at child age 4.5 were associated with child ADHD symptoms at age 6. This finding provides information about the heritable processes whereby children shape their environments, and how those processes can impact children's risk for psychopathology. The prevention and developmental implications are two-fold: First, the parent-offspring adoption design allowed for detection of the salience of hostile maternal parenting behavior on children's ADHD symptoms, while eliminating the effects of passive *r*GE. Second, this design permitted the detection of the role of early disrupted child behavior (impulsivity/activation) as a mechanism through which genetically-influenced child attributes influence their rearing mothers' hostility, which in turn

predicted children's later ADHD symptoms. As such, two pathways to prevention are identified: via interventions that aim to reduce hostile maternal parenting behavior, and via interventions that coach mothers to identify children's early disruptive behavior and respond in constructive ways that break evocative patterns.

Naturalistic Experimental Design 2: Siblings Reared Apart

The second naturalistic experimental design that has been applied to humans is the siblings reared apart design. Drawing from the plant and animal literature, this approach can be characterized as a naturalistic cross-fostering design. In this paradigm, a newborn is removed from the biological parent home around the time of birth and is reared by genetically unrelated parents. In addition, the biological parent is parenting a biological child, who is the sibling to the child reared in the adoptive home. The unique feature of this design is that while genetic influences on a phenotype are shared among siblings (because they have the same biological mother and/or father), the rearing environment to which each sibling is exposed differs (See Figure 1). In non-human animal and plant studies, the cross-fostering approach is considered one of the gold standard research paradigms for studying the effects of the rearing environment and gene-environmental interplay (e.g., Meaney, 2001). This is because the cross-fostering design allows clear separation of environmental and genetic effects and, thus, examination of the interplay between the two on resulting behaviors is feasible. However, for obvious ethical reasons, the application of randomized cross-fostering designs to human populations is limited.

Nonetheless, a few research groups have creatively applied the concepts underlying the cross-fostering design to naturally occurring phenomena in humans, using natural experimental designs (Rutter, Pickles, Murray, & Eaves, 2001). The first type of natural cross-fostering approach is the twins reared apart design. The Minnesota Study of Twins Reared Apart

(Bouchard, Lykken, McGue, Segal, & Tellegan, 1990) and the Swedish Adoption/Twin Study of Aging (Pedersen et al., 1991) are the two best-known studies in this category. In both studies, each twin was reared in a separate adoptive home, and interviewed as adults. The major strength of the twins reared apart design is that it controls genetic similarities across twin pairs, especially in the case of monozygotyic (MZ) twins. This design is powerful because the MZ twins share genes but not environments, and it can thus be assumed that differences in the behaviors of the MZ twin pairs are likely due to environmental influences. A second type of human study extends the twins reared apart design by incorporating non-twin siblings who were placed in separate adoptive homes. One existing study examined siblings' criminal behavior and found that conviction rates of siblings were similar despite the fact that they were reared apart, especially when their biological father had a court conviction (Mednick, Gabrielli, & Hutchings, 1984).

In a third cross-fostering research paradigm used with humans, adoptees placed at birth are compared to their biological siblings who remained in the home of origin and who are thus reared by their biological parents. In this design, siblings who remain in the home of origin serve as a quasi-reference group that can provide information about potential outcomes had an individual not been adopted. Examples of this type of cross-fostering design include a Danish adoption study of obesity (Sorensen et al., 1989), a small sample of French children (n = 20 sibling pairs; Schiff et al., 1982), and a study of adult full- and half-sibling pairs compiled using multiple Swedish nationwide registries where at least one sibling was reared by one or more biological parents and the other by adoptive parents (Kendler et al, 2016). In the Kendler et al. (2016) study, results indicated that the adopted siblings had a substantially lower risk for drug abuse than the biologically-reared siblings, suggesting environmental influences on drug abuse.

The EGDS Siblings Reared Apart Study. A new siblings reared apart study is

underway that extends the EGDS parent-offspring adoption study (discussed earlier) by assessing the biological siblings of EGDS adoptees. Of the extant siblings reared apart designs, this study is uniquely aligned to inform prevention research because the family environment was measured in both birth and adoptive family homes in detail, beginning in early childhood. In this study (Early Parenting of Children Study; EPoCh), a subset of the EGDS biological parents are parenting their own biological child(ren). These families are recruited into EPoCh, with $n = 142$ families recruited and assessed to date. The EGDS adoptees and their biological siblings have been reared apart since birth, never sharing the same postnatal rearing environment. When a child in either home turns age 7, the study team conducts an in-depth assessment of their rearing environment, child behavior, and prenatal events (e.g., exposure to toxins, substances, stress). When data collection is complete, there will be equivalent measures of the rearing environment and child behavior at age 7 for the adoptee and the sibling reared by the biological parent(s).

Prevention Relevant Results. To document the utility of the siblings reared apart cross-fostering design to identify environmental influences on child psychopathology, we examined the rearing environments of the two households (adoptive home and biological home) in EPoCh. As shown in Table 1, all parent, home, and neighborhood environment characteristics were significantly different between the adoptive and biological parent family, suggesting that the siblings reared apart experience very different rearing environments from one another. For example, there is about a threefold difference in the annual household income between the two households. Likewise, the sibling living in the biological mother's home is reared by a mother with an official criminal record 62% of the time, whereas none of the siblings reared in the adoptive homes have this environmental exposure. Notably, the reared-apart siblings did not differ significantly on pregnancy-related variables, such as child birth weight or fetal alcohol

syndrome facial features (Table 1). This is important because it suggests that the prenatal conditions of the siblings reared apart may be comparable, even when the postnatal environments are not, which allows for greater confidence that any sibling behavioral differences are likely specific to postnatal, rather than prenatal, environmental influences. The striking differences in rearing environments of the siblings allows for analyses that control for the effects of shared genes while identifying environmental features that may lead a sibling in one home to develop problems but lead a sibling in the other home to remain symptom free.

The ability to separate the effects of the rearing environment from the effects of shared genes between parent and child could inform targeted intervention efforts to prevent child psychopathology. Siblings reared apart designs that include highly specified measures of both biological and adoptive rearing environments can identify discrete family processes that show associations with child behavior. They can also identify family processes that offset or exacerbate inherited risk. Due to the fact that most prior family process studies sample biological families, a clear delineation of environmental influences free from the confounding of genetic influences is typically not possible without a genetically-sensitive design such as this. The specific family processes identified in siblings reared apart designs can therefore provide new insights into malleable aspects of the environment that could be targeted in prevention studies.

Naturalistic Experimental Design 3: The *In Vitro* Fertilization (IVF) Design

The IVF design is a third natural experimental design that has been made possible by medical advances over the last several decades. Its design is consistent with prenatal cross-fostering approaches used in the life sciences. Assisted reproductive technologies such as IVF are an increasingly common means of conception, and it is estimated that more than 5 million children have been born via IVF since 1978 when the first IVF baby was born

(https://www.eshre.eu/Guidelines-and-Legal/ART-fact-sheet.aspx). This phenomenon has created a new opportunity to examine whether associations between parenting and child behavior might vary as a function of whether the child is genetically related to both, one, or neither parent.

There are several different constellations of genetic and prenatal relatedness when children are conceived through IVF methods. First, in homologous IVF, children are genetically related to both of their rearing parents, with the egg coming from the rearing mother and the sperm from the rearing father but conception occurring through artificial reproductive technologies. Second, in sperm donation, the child is genetically related to the rearing mother but not the rearing father. Third, in egg donation, the child is genetically related to the rearing father but not the rearing mother. Fourth, in embryo donation, the child is genetically unrelated to both rearing parents. Fifth, in one form of gestational surrogacy, the child is genetically related to both rearing parents, but the intrauterine environment is provided by a genetically unrelated surrogate.

By comparing the association between a parental variable and a measure of child psychopathology between dyads who are genetically related (mothers: homologous IVF, sperm donation, surrogacy; fathers: homologous IVF, egg donation, surrogacy) and genetically unrelated (mothers: egg and embryo donation; fathers: sperm and embryo donation), this natural experimental design approach can be used to examine whether the magnitude of the association between parent and child is primarily genetically mediated, environmentally mediated, or a combination of the two. For example, when an association is identified between rearing parent depression and child depression among genetically related parent and child dyads, but not between genetically unrelated parent and child dyads, the association is attributable to genetic mediation. When the association between rearing parent and child depression is present among genetically related and genetically unrelated dyads, the association cannot be entirely genetically

mediated and environmental mechanisms must be influencing the association. Furthermore, similar to the adoption design described earlier, when significant associations are found among genetically unrelated parent-child dyads (i.e., where passive *r*GE is absent), the primacy of environmental mechanisms underlying this association is apparent. Last, by comparing parent-child associations between homologous IVF families and children born through surrogacy, because the former share genes, prenatal environment, and rearing environment whereas the later share only genes and rearing environment, prenatal environmental influences can be isolated. These between family differences permitted by examining IVF variations in genetic and prenatal relationship have high relevance for the design of preventive interventions, as discussed below.

The Cardiff IVF Study. The largest psychosocial study of children born through IVF is the Cardiff IVF study. Families who had a live birth following successful artificial reproductive treatment from any of the five conception groups described above were recruited from 19 clinics (18 UK clinics and one US clinic; Thapar et al., 2007). This study required that gamete donors and surrogates were unrelated to either rearing parent. Data were collected by mailed questionnaires sent to families by each participating clinic. Children were aged between 4-11 years (M = 6.8 years, SD = 1.24) at the time of assessment. The number of families in each conception group is: 444 homologous IVF, 210 IVF with sperm donation, 175 IVF with egg donation, 36 IVF with embryo donation, and 23 IVF with gestational surrogacy.

Prevention Relevant Results. The Cardiff-IVF sample has been used as a natural experimental design to examine the effects of parenting and parental psychopathology on child adjustment, and to examine the role of the prenatal environment. The results and prevention implications of two Cardiff IVF studies are described here. In the first study, the authors examined the intergenerational transmission of antisocial behavior and depression by comparing

parent-child associations across genetically related versus genetically unrelated parent-child dyads and examining the mediating role of parental hostility to the child (Harold et al., 2011). For antisocial behavior, path analyses indicated direct associations between parent antisocial behavior and child antisocial behavior that were fully mediated by parent-to-child hostility for both genetically related and genetically unrelated groups. This pattern of results was consistent for mothers and fathers. The similar pattern of results for genetically related and unrelated dyads highlights the role of parent-to-child hostility as an environmental risk mechanism for the transmission of antisocial behavior. In comparison, for depression, path analyses indicated a direct association between parent depression and child depression for genetically related mothers and fathers that was partially mediated by parent-to-child hostility for fathers and fully mediated for mothers; however, there was no evidence of mediation for genetically unrelated mothers or fathers (and an absence of a direct path between father depression and child depression in genetically unrelated dyads). This lack of association between parent-to-child hostility and child depression for genetically unrelated dyads, but the presence of this association for genetically related dyads, suggests the likely role of shared genes in influencing the link between parental hostility and the child's depressive symptoms. Taken together, the pattern of results in this study suggests that the role of genes may be more important in the intergenerational transmission of depression than antisocial behavior, and that parent-to-child hostility is an important environmental mechanism that could be targeted in intervention studies to help mitigate risk.

In a second study, the Cardiff IVF sample was used to examine the association between maternal smoking during pregnancy and child antisocial behavior (Rice et al., 2009). The inclusion of the genetically unrelated rearing mothers (embryo donation or egg donation) provides a naturalistic experiment to help disentangle genetic from prenatal associations.

Analyses indicated that the association between prenatal smoking and child antisocial behavior was present in genetically related mother-child dyads, but was absent in genetically unrelated mother-child dyads. This suggests that genetic factors play a role in the association between maternal smoking during pregnancy and child antisocial behavior, rather than there being a direct causal pathway from prenatal smoking to child antisocial behavior. Conversely, this study found that prenatal smoking reduced child birth weight in both unrelated and related children, consistent with effects arising from prenatal mechanisms independent of the relation between the maternal and child genomes. This design and pattern of findings can thus point prevention researchers in the direction of specific postnatal and/or prenatal intervention targets in ways that would not be possible without this type of design.

Limitations of Naturalistic Experimental Designs in Humans

Although the three naturalistic experimental designs described in this article represent novel approaches to building on the rigor of quantitative genetic methods from the life sciences, they are not without limitations. For example, if selective placement or matching of adoptive and birth parents is present, or if adoptees have ongoing contact with their birth parents, the design assumptions in the parent-offspring adoption design are violated and associations between birth parents and adoptees might not only include genetic and prenatal influences, but may also include postnatal environmental influences. In addition, it can be difficult to determine the best proxy of an adult trait in a young child. For example, when examining children's heritable risk for antisocial behavior by studying criminal behavior in the birth parents, what is the best measure of inherited risk for antisocial behavior in a 4-year old child? The science in the selection of these variables is far from precise at this time. Similarly, in the siblings reared apart cross-fostering design, it is important to consider potential "third" variables. The many

environmental differences between adoptees and their biological siblings pose an unavoidable third variable threat. Further, because couples who adopt are screened by agencies for financial security, but no such screening is applied to biological parents who chose to parent, there are unavoidable economic and other differences between birth parents and adoptive parents that make a full cross-fostering design unattainable. Finally, in the IVF design, prenatal effects cannot be disentangled from postnatal effects without inclusion of the surrogacy group, which was small in the Cardiff IVF study (n = 23). In addition, unlike the parent-child adoption design and the cross-fostering design, the IVF design does not include measurement of characteristics of the donor parent (i.e., the genetically-related parent). Thus, the designs described in this article are quasi-experimental, naturalistic approaches that are subject to a lack of precision, but that allow for novel insights into the role of the environment in influencing children's risk for psychopathology that could not be discerned without genetically-sensitive methods.

New Directions in Genetic Research with High Prevention Relevance: The Microbiome

There are several new frontiers in gene-environment interplay research with potential relevance for prevention science (e.g., recent work on epigenetic effects). One such frontier is the study of the human microbiome. The microbiome is the collection of microorganisms (and their genes) associated with a particular animal or plant host. There has been an explosion of research regarding microbiomes in recent years (see Cho & Blaser 2012 for a review). Recent research, for example, has shown that the composition of the human microbiome varies across individuals and can be as distinctive as a fingerprint (Franzosa et al., 2015) and that humans emit a "cloud" of microbes that can potentially result in transfer of microbes across individuals (Meadow et al., 2015). The composition of the human microbiome has been linked to a number of important human physiological traits, including effective digestion and maturation of the immune system,

as well as the incidence of important health disorders such as asthma, inflammatory bowel disease, and obesity (Cho & Blaser, 2012). The human microbiome has also been recently linked to sleep patterns, mood, and other behaviors (Foster & Neufeld, 2013) and may play a role in the development of behavioral disorders such as autism (Mulle et al., 2013). In recognition of our growing understanding of the importance of microbiomes, the White House recently announced a $500 million investment in the National Microbiome Initiative (https://www.whitehouse.gov/blog/2016/05/13/announcing-national-microbiome-initiative).

Despite the recognized importance of the human microbiome, it is not yet clear how humans acquire their associated microbes, nor is it understood what underlies variation in microbiomes across individuals. It is also not clear how the microbiome interacts with the human genome and the external environment to determine human phenotypes. The microbiome has often been referred to as a "second genome" (e.g., Grice & Segre 2012), and as such, one could think of microbiome influences on phenotypes as a type of "genetic" effect (the G in G x E). However, this is not completely true; the microbiome of an individual is more variable and more dynamic than an individual's genome, and its composition is more sensitive to the surrounding environment than that of the genome (e.g., Franzosa et al., 2015). In this sense, the microbiome can be thought of as part of the "environment" (the E in G x E).

Given the potential importance of the microbiome in human disorders central to the study of prevention science (such as behavioral disorders and obesity), understanding the interplay of the human genome, microbiome, and environment in influencing human traits is an important topic for prevention science research. The naturalistic experimental designs described in the preceding sections could be used to ask fundamental questions about this interplay by collecting and analyzing microbiome samples in adoption, cross-fostered, or IVF samples. Several recent

studies have used twin designs to ask questions regarding the importance of genetics in determining the composition of the human microbiome (e.g., Goodrich et al. 2016), but the use of natural "common garden" or "cross–fostering" approaches in human populations to partition the effects of genetics, the microbiome, and the environment and examine their interaction would add to the field of prevention science by helping to identify specific environmental attributes that influence the microbiome and potentially offset inherited health risks.

Measurement of the Environment

Effective translation of findings from naturalistic experimental studies such as the adoption design, the siblings reared apart design, and the IVF design to prevention research depends in large part on the alignment of the conceptualization and measurement of the environment across the design types. For example, in the adoption study finding presented earlier (Leve et al., 2009), the environment was operationalized as parental structure. This is akin to Baumrind's control dimension that is a component of authoritative and authoritarian parenting styles (Baumrind, 1971). However, this operationalization does not include the warmth dimension of Baumrind's parenting typology, and as such, qualities related to maternal emotional tone are unmeasured, and a distinction between authoritative and authoritarian parenting is not possible. In addition to structure, parenting qualities such as warmth, positive support, and sensitivity are important for healthy child development and have been shown to be key behavioral targets in prevention studies (e.g., Lunkenheimer et al, 2008). The lack of harmonization between the conceptualization and measurement of parenting in a qualitative genetic study and that in a prevention study can pose a barrier to effective translation.

Similarly, the "environment" is a very broad concept that not only includes dimensions of parenting, household milieu, and neighborhood that are common foci in prevention studies, but it

also includes qualities of the physical environment such as allergens, pollutants, toxins, and temperature/climate. Drawing upon the foundation of plant and non-human animal research described earlier, these physical dimensions of the environment have known effects on the health and outcomes of living organisms. Further, such exposures can be readily modified via human intervention. A recent NIH initiative on environmental influences on child health outcomes (https://www.nih.gov/echo) focuses in part on these physical aspects of the environment, with plans to apply the results of this work to inform clinical trials. In order for the potential translation from naturalistic experimental designs to prevention research to be realized, it is important that future studies are designed with an eye toward aligning the conceptualization and measurement of the environment across the disciplines.

The Urgency of Pursuing Genetically-Informed Prevention Research with Naturalistic Experimental Designs

The idea that some day we may be able to leverage personalized, genetic approaches to promote health and well-being has sparked interest from researchers, medical providers, and patients alike. For example, President Obama discussed the promise of tailored medical treatments and a "precision medicine" approach in his 2015 State of the Union address. This initiative, which includes the recruitment of a volunteer national research cohort, may help pave the way for clinicians to apply individualized treatments based on a patient's biology, as guided by genetic information collected from saliva or blood in medical settings. Simultaneous to these biomedical efforts, we advocate that a quantitative genetic approach using naturalistic experimental designs such as those described in this article provides an alternative pathway to precision medicine.

For example, through the use of cross-fostering designs, we can learn how genetically-

related siblings reared apart in separate home environments may or may not develop the same disorders, and which aspects of their respective rearing environments are linked to these differences. The IVF design can help identify whether an environmental correlate (e.g., maternal smoking) of child behavior problems is present because of a child's prenatal exposure, postnatal exposure, or both. And the parent-offspring adoption design can separate genetic and prenatal risks from postnatal risks on this same child phenotype. Together, these designs can increase our understanding how inherited characteristics of an individual can affect the extent to which a specific therapy or intervention related to the prevention of psychopathology is beneficial.

How might such a genetically-informed preventive intervention trial look? The first steps in such an endeavor are to critically examine the evidence base on both the quantitative genetic research side and the preventive intervention research side. On the quantitative genetic side, prevention scientists should attempt to conduct studies that: 1) specify a theory of change, 2) examine the role of heritable and environmental influences on individual outcomes using robust conceptualization and measurement of the environment and the individual outcome, in ways that map onto the environmental targets and the individual outcomes of a particular preventive intervention, 3) are replicable, reproducible, or can otherwise be shown to represent a robust effect on the interplay between inherited and environmental influences. In parallel to these efforts, on the intervention side, prevention scientists should: 1) identify (or develop) an intervention that maps onto a similar theory of change as the quantitative genetic study defined above, 2) ensure that there is a specific environmental mediator that can be targeted via intervention and that has overlap with the measurement of the environment in the quantitative genetic study, and 3) if it is an existing intervention, identify for whom and under what conditions the intervention has been shown to be effective. These steps will help link the

quantitative genetic findings with the mechanisms of change in the intervention so that the intervention can be thoughtfully modified (or designed, if a new intervention) to take into account the role of heritable characteristics on the intervention's mechanism of change.

Next, because each of the genetic designs described here leverages data from genetically-related family members to quantify heritable risks and protective factors, an important next step is to collect detailed information about biological and non-biological family members' psychopathology. Based on this information, children could be categorized in terms of their level of inherited risk. Drawing from the findings on structured parenting described earlier (Leve et al., 2009), families could then be randomized to receive services as usual or a tailored intervention designed to match the child's level of inherited risk. Specifically, where children had low inherited risk, intervention parents who were over-structuring could be taught strategies such as providing positive reinforcement in lieu of over-structuring; where children had high inherited risk, parents could be taught specific ways to provide additional structure to their child's activities. If this precision approach were effective, intervention children at high and low inherited risk would both show a reduction in behavior problems, as compared to children in the control condition.

Such an approach is not without challenges, including the need to design an intervention that could be delivered remotely (e.g., via web or app interface) in order to reach a geographically dispersed population. Further, there are limits to the level of malleability attainable, as documented in studies of children reared in orphanages who were subsequently adopted. Nonetheless, given the potential of personalized medicine approaches to spur new discoveries, there has never been a better time to integrate naturalistic experimental designs such as those described in this article into the burgeoning world of personalized medicine approaches.

Compliance With Ethical Standards

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Ethical Approval: This is a review article and no original research was conducted for this manuscript. The original studies described in this manuscript that were led by the current authors (EGDS, EPoCh, and Cardiff IVF Study) received approval from their respective Institutional Review Boards. All procedures performed in these three studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This article does not contain any studies with animals performed by any of the authors.

Informed Consent: No original research was conducted in the course of this work. Informed consent was obtained from all individual participants included in the three studies described in this manuscript that were led by the current authors (EGDS, EPoCh, and Cardiff IVF Study).

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Table 1. A Comparison of Adoptive and Biological Family Environments (Mean or Percent and (SD)).

	Adoptive Families	**Biological Families**
Rearing mother anxiety disorder	25%	52%*
Rearing father anxiety disorder	16%	44%*
Rearing mother substance use disorder	25%	59%*
Rearing father substance use disorder	35%	72%*
Median annual household income	$100,001–125,000	$25,001–40,000*
Median education level	4 yrs college	High school degree*
Rearing mother age at birth of target child	37.58 (5.7)	24.35 (5.1)*
Marital status (% 2-parent household)	87	50*
Rearing mother WAIS vocabulary scaled score	11.44 (2.4)	9.48 (3)*
Rearing mother official criminal record (% yes)	0	62*
Rearing mother body mass index (BMI)	25.02 (5.4)	27.04 (6.8)*
Rearing mother currently smoke cigarettes regularly (% yes)	4	46*
Neighborhood quality[a]	1.28 (0.4)	1.9 (0.6)*
Child birth weight (pounds) [prenatal env]	7.2 (1.2)	7.12 (1.4)
FAS 4-digit diagnostic code [prenatal env]	1.68 (0.7)	1.93 (0.7)

Note. [a]Higher value = poorer quality; * $p < .001$

Figure 1. The Role of Naturalistic Experimental Designs in Guiding Genetically Informed Intervention Targets.